Express Mail No. EV 336618751US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Christen M. Anderson, et al.
Application No.:	09/185,904
Filed:	November 3, 1998
For:	PRODUCTION OF ADENINE NUCLEOTIDE TRANSLOCATOR (ANT), NOVEL ANT LIGANDS AND SCREENING ASSAYS THEREFOR

Examiner	:	Holly G. Schnizer
Art Unit	:	1653
Docket No.	:	660088.420
Date	:	November 4, 2003

DECLARATION Of CHRISTEN M. ANDERSON, M.D., PH.D. UNDER 37 C.F.R. § 1.132

Mail Stop RCE
Commissioner for Patents
P. O. Box 1450
Alexandria. VA 22313-1450

I, Christen M. Anderson, a citizen of the United States, declare and state that:

1. I was a Scientist and Director of Metabolic Diseases at MitoKor, Inc., San Diego, California, United States, the assignee of the above-identified patent application (hereinafter referred to as "instant application"), at the time of filing, and I am a co-inventor on the instant application.

2. I have read the Office Action dated January 8, 2003, including the rejections of claims 42, 44-52, 56 and 57 of the instant application under 35 U.S.C § 103.

3. Prior to the invention described in the instant application, there was a long-felt, unmet need in the art for recombinantly produced and isolated human ANT3 polypeptides and fusion proteins, particularly those that retained functional properties, including the ability to bind human ANT3 ligands. In addition, the successful production and isolation of recombinant human ANT3 polypeptides and fusion proteins had not been described at the time of filing the instant application, although numerous failed attempts had been made. My conclusions are based, in part, upon the evidence described below.

4. The skilled artisan would readily appreciate that there was a need in the art for recombinantly produced and isolated human ANT3 polypeptides and fusion proteins, given the fundamental role of ANT polypeptides in mitochondrial function, including human oxidative phosphorylation. It was known for some time that ANT polypeptides play a prominent role in respiratory, metabolic, apoptotic and other processes of the mitochondria, the altered function of which, in turn, is thought to be associated with numerous metabolic degenerative diseases. The skilled artisan would appreciate that in order to characterize the functional and structural properties of ANT3 polypeptides at the molecular level, to further understand their role in human disease, and potentially to identify therapeutic compounds suitable for treatment of mitochondrial-based disease, a reliable source of functional ANT3 polypeptides and fusion proteins is needed.

5. Furthermore, the skilled artisan would recognize that the need for recombinant human ANT3 polypeptides and fusion proteins was long-felt, since the important physiological role of ANT polypeptides was recognized as long ago as the early 1980s (*e.g.,* Klingenberg, M. (1981) *Nature* 290, 449-454), yet recombinantly produced human ANT3 polypeptides and fusion proteins were not described before the instant application of 1998. Indeed, the attention directed to human and animal ANT polypeptides by numerous investigators, as evidenced by references cited throughout the instant application (*see, e.g.,* page 15, lines 15-26; pages 39-40; Fiore *et al.* (1998)

Biochimie 80, 137-150), and elsewhere, clearly demonstrates the recognition in the art of a compelling need for a consistent, readily-produced and reliable source of ANT polypeptides.

6. Further, this long-felt, unmet need for recombinantly produced and isolated human ANT3 polypeptides and fusion proteins provides evidence of the failed attempts by others to produce these polypeptides. The cDNA sequence encoding human ANT3 was known as early as 1988 (Houldsworth, J. and Attardi, G. (1988) *Proc. Natl. Acad. Sci U.S.A.* 85, 377-381), and recombinant protein expression methods were known well before this date. Given the established need for recombinantly produced human ANT3 polypeptides and fusion proteins, the skilled artisan would immediately conclude that attempts to produce such polypeptides using known techniques had been made. It readily follows that the lack of any report or publication describing the successful production of recombinant human ANT3 polypeptides indicates that these attempts were unsuccessful.

7. In addition, direct evidence of failed attempts to produce and isolate recombinant human ANT polypeptides and fusion proteins is provided in numerous publications, including those described below.

(a) Fiermonte *et al.*, (1993) *Biochem. J.* 294, 293-299, describes failed efforts to express and reconstitute functional ANT polypeptides from bacteria. Fiermonte *et al.* illustrate some of the numerous difficulties associated with the expression and isolation of functional mammalian ANT polypeptides, including low yields due to mammalian ANT toxicity to host cells and the inability to isolate functional recombinant mammalian ANT polypeptides. Fiermonte *et al.* describe attempts to express and isolate two mitochondrial membrane transport proteins, the oxyglutarate carrier and a mammalian ANT polypeptide. Although Fiermonte *et al.* were able to express significant levels of the oxyglutarate carrier, they were only able to express low levels of non-functional ANT due to the toxicity of the ANT polypeptide in the bacterial cells. In particular, while Fiermonte *et al.* were able to isolate and reconstitute functional oxyglutate, they were unable to isolate functional ANT polypeptides. This is indicated in their statement "[t]he expression of the ADP/ATP carrier in bacteria is also a step toward

[an opportunity to study which amino acids are essential for the function of the carrier by site-directed mutagenesis], although the essential reconstitution step has not yet been achieved." (emphasis added) (see Discussion, page 298). Clearly, this reference provides evidence of the failure of others to reconstitute a functional ANT polypeptide. Furthermore, this reference provides evidence of the difficulties specific to producing recombinant ANT polypeptides, as compared to other mitochondrial membrane transport proteins. Accordingly, this reference demonstrates unsuccessful attempts by others to produce isolated recombinant mammalian ANT polypeptides and further demonstrates that the production of recombinant ANT polypeptides was not routine, and that methods of doing so were not obvious to the skilled artisan.

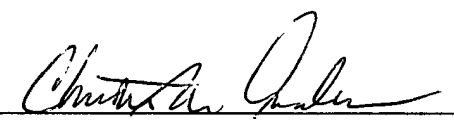
(b) Further evidence of the failure of others to produce recombinant ANT protein is provided in Miroux *et al.* (1996) *Journal of Molecular Biology* 260(3), 289-298. Miroux *et al* describe multiple problems with regard to efforts to express recombinant ANT, including toxicity to host cells, poor solubility of the recombinant product, and the accumulation of recombinant ANT in inclusion bodies. The skilled artisan would appreciate the persistent and challenging problems associated with isolating functional polypeptides from inclusion bodies. Extracting insoluble protein under non-denaturing conditions using detergents and sonication is time consuming and yields low levels of protein, and cannot always be expected to result in a functional protein. Extracting a protein under denaturing conditions with urea or other reagents is often successful, but using these methods requires refolding the protein and frequently results in a loss of structure and activity. Based upon this understanding, I submit that the isolation of functional ANT polypeptides from inclusion bodies is not routine, and the skilled artisan would have no reasonable expectation of being able to successfully isolate functional ANT polypeptides based upon Miroux *et al.*'s teaching that ANT polypeptides accumulate in inclusion bodies.

(c) Rojo and Wallimann ((1994) *Biochim et Biophysica Acta* 1187, 360-367) describe efforts to purify ANT polypeptides from tissue using various detergents, and demonstrate the difficulties associated with purifying and reconstituting functional ANT polypeptides. Rojo and Walliman note that "[a]lthough various

detergents have been the subject of systematic studies concerning multiple aspects of their interaction with membranes and membrane proteins, still no rationale exists that allows us to identify *a priori* the detergent or detergent class that is suited for a particular protein and/or application. Thus, detergents have to be selected based upon the basis of empirical studies." This reference indicates that methods successful in the purification or isolation of ANT polypeptides are unpredictable, and the skilled artisan would, given the state of the art at the time of filing the present application, have had no reasonable expectation of successfully isolating functional ANT polypeptides using any particular method. Furthermore, this reference provides additional evidence establishing the unpredictability in isolating functional polypeptides from inclusion bodies.

8. In conclusion, I submit that the above-cited references provide evidence of a long-felt need for and the failure of others to successfully express, purify and reconstitute recombinant ANT polypeptides and fusion proteins. In addition, I submit that these references clearly indicate that the production of functional ANT polypeptides and fusion proteins is not routine and that without the teaching of the present application, the skilled artisan would have no reasonable expectation of successfully producing and isolating ANT polypeptides.

9. The undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.



Christen M. Anderson, M.D., Ph.D.

11/1/03

Date